[redacted]

APPENDIX 2 -- EXHIBITS

L32 ANSWER 45 OF 72 CANCERLIT
ACCESSION NUMBER: 94690969 CANCERLIT
DOCUMENT NUMBER: 94690969
TITLE: The effect of copper and **gallium** compounds on **ribonucleotide reductase**.
AUTHOR: Narasimhan J
CORPORATE SOURCE: Medical Coll. of Wisconsin.
SOURCE: Diss Abstr Int [B], (1993) 53 (10) 5114.
ISSN: 0419-4217.
DOCUMENT TYPE: (THESIS)
LANGUAGE: English
FILE SEGMENT: Institute for Cell and Developmental Biology
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19941107
Last Updated on STN: 19970509

ABSTRACT:
The mode of action of copper complexes (CuL and CuKTS) and **gallium** compounds (**gallium** nitrate and citrate) in cytotoxicity was studied. The effects of these agents on the enzyme **ribonucleotide** ***reductase*** was investigated by monitoring the tyrosyl free radical present in the active site of the enzyme through electron spin resonance spectroscopy. **Ribonucleotide reductase** is a key enzyme in cellular proliferation since it catalyzes the conversion of ribonucleotides to deoxyribonucleotides, the precursors in DNA synthesis. In consists of two subunits namely M1 and M2. M1, a dimer of molecular weight 170,000, contains the substrate and effector binding sites. M2, a dimer of molecular weight 88,000, contains non-heme iron and tyrosyl free radical essential for the activity of the enzyme. In the studies using copper complexes, the cellular oxidative chemistry was examined by ESR studies on adduct formation with membranes, and oxidation of thiols. Membrane thiols were shown to be oxidized through the reduction of the ESR signal of the thiol adduct and the analysis of sulfhydryl content. Using the radiolabel 59Fe, the inhibitory action of copper thiosemicarbazones on cellular iron uptake was shown. The inhibitory action of CuL on **ribonucleotide reductase** was shown by the quenching of the tyrosyl free radical in the M2 subunit. The hypothesis that ***gallium*** directly interacts with the M2 subunit of the enzyme and displaces the iron from it was proven to be true. The tyrosyl free radical signal from cell lysates was shown to be inhibited by the direct addition of ***gallium*** compounds. Furthermore, the signal was regenerated upon addition of soluble iron to the cell lysates. **Gallium** content in the cells was measured by a fluorimetric method, to ensure the presence of sufficient amounts of **gallium** to compete with the iron in the M2 subunit. The enzyme activity, measured by the conversion of [14C]-CDP to the labeled deoxy CDP, was shown to be inhibited by the addition of **gallium** nitrate in a cell free assay system. The immunoprecipitation studies of the 59Fe-labeled M2 protein using the monoclonal antibody directed against this subunit suggested that **gallium** releases iron from the M2 subunit.
(Full text available from University Microfilms International, Ann Arbor, MI, as Order No. AAD93-02808)

CAS REGISTRY NO.: 7440-50-8 (Copper); 9007-49-2 (DNA); **7440-55-3 (Gallium)**
CHEMICAL NAME: 0 (Free Radicals); EC 1.17.4 (**Ribonucleotide**

Reductases); 0 (Sulfhydryl Compounds)

=>